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Inclusion complex between β-cyclodextrin and hecogenin acetate produces superior analgesic effect in animal models for orofacial pain

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ABSTRACT

Hecogenin acetate (HA) is a steroidal sapogenin-acetylated with pharmacological properties which have already been described in the literature such as, anti-inflammatory, anti-hyperalgesic and antinociceptive, but it has low solubility in aqueous media. Therefore, in an attempt to overcome this, we set out to create inclusion complexes between HA and b-cyclodextrin (b-CD) and evaluate the antinociceptive effects in the orofacial nociception in mice. The complexes were prepared using different methods in the molar ratios 1:1 and 1:2 and characterized physicochemically. The results of the physicochemical characterization elucidated inclusion complexes formation between b-CD and HA by freeze drying method in the molar ratio 1:2, which obtained a complexation efficiency of 92% and produced superior analgesic effect in animal models for orofacial pain at a lower dose when compared to HA alone.

1. Introduction

Orofacial pain represents a major medical and social problem in the United States and has been demonstrating a steady increase over the last several decades [1]. Its pathophysiology and cause is related to cranial neuralgia, headache, dental pain, temporomandibular disorders and persistent idiopathic facial pain; referring to pain associated with to the soft and hard tissues of the head, face and neck [2]. Currently, the pharmacological treatments used such as nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, peripheral analgesic, antidepressant drugs and corticoids present few effectiveness due to non-specificity and pharmacoresistance [3]

In this sense, the natural products (NPs) has been a promising alternative for new chemical entities with possible applicability in orofacial pain, hoping to discover new biologically active substances that may offer news possibility of treatment, since there is no pharmacological treatments which may result in clinical improvement without significant side effects in these pathological conditions [4]. Hecogenin is steroidal saponin, found in the leaves of species from the *Agave* genus, used by pharmaceutical industry in the production of oral contraceptives [5,6].

Moreover, hecogenin acetate (HA) is a steroidal saponin-acetylated with analgesic property, acting in descending pain-inhibitory mechanisms through its action on the opioid receptors (mainly in central nervous system) and reduction of pro-inflammatory cytokines, as IL-1β [7,8]. Presents poor solubility in water and the use of β-cyclodextrin (β-CD) seems to be promising for produce improvements in solubility and bioavailability of compounds non-polar, because have suitable molecular

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dimension of cavity (diameter 0.60–0.65 nm) which provides a mean to stabilize intramolecular complexes with a wide variety of guests, including drugs and hydrocarbon amphiphiles [9–12]. Quintans et al., 2016 showed that oral pretreatment with HA/β-CD presented antinociceptive profile and also decreased mechanical hyperalgesia significantly when compared to HA alone, suggesting that β-CD improved the anti-hyperalgesic effect of HA [13].

Considering low solubility in water and both the pharmacological potential this saponin, the present study had aims to elucidate the influence of the molar ratio and the method of preparation on the formation of inclusion complexes between β-CD and HA, as well as, evaluate the antinociceptive effect in the orofacial nociception in mice.

2. Material and methods

2.1. Material

HA (purity ≥ 90%), β-CD (purity ≥ 97%), monosodium glutamate (USP Reference Standard) and capsaicin (≥ 95%, from *Capsicum* sp), were purchased from Sigma-Aldrich (USA). Acetic acid was purchased from Vetec (Brazil).

2.2. Preparation of samples

The samples were prepared based on HA's molecular weight (472,66 $gmol^{-1}$) and β-CD's molecular weight (1134,98 $gmol^{-1}$), with a molar ratios of 1:1 and 1:2 (HA:β-CD) by methods of physical mixture (PM), paste complexation (PC), freeze drying (FD) and filtration (FI).

2.2.1. PM

The PM sample was prepared by addition HA to an agate mortar containing β-CD powder under manual stirring, which were then stored in sealed glass containers [14].

2.2.2. PC

The PC sample was prepared by addition of 2 ml of distilled water to a mixture of HA and β-CD, directly in an agate mortar and subsequently kept under constant manual agitation. The material was then dried at room temperature (in a desiccator) until a clear film was formed, which was removed by manual trituration and stored in airtight glass containers [14].

2.2.3. FD

The FD sample was prepared by addition of HA and β-CD to 20 ml of distilled water, this solution was submitted to agitation by a magnetic stirring device operating at 400 rpm (Quimis Q 261A21, Brazil) at room temperature for 36 h, then was frozen at −72 °C for 4 h and freeze-dried. After, was stored in airtight glass containers. This method of preparation was adapted from Menezes et al., 2012 [14].

2.2.4. FI

The FI sample was prepared by addition of HA and β-CD to 20 ml of distilled water, this solution was submitted to agitation by a magnetic stirring device operating at 400 rpm (Quimis Q 261A21, Brazil) at room temperature for 36 h, was vacuum filtered (filter paper 150 mm) and stored in a desiccator at room temperature until all moisture was removed. After, was stored in airtight glass containers. This method of preparation was adapted from Menezes et al., 2012 [14].

2.3. Characterization of the complex

2.3.1. Thermal analysis

The DSC and TG/DTG curves were obtained in the temperature range of 30 to 500 °C and 30 to 900 °C respectively using Shimadzu DSC-60 and TGA-60 instruments under dynamic nitrogen atmosphere (100 $ml.min^{-1}$) and a heating rate of 10 °C/min utilizing aluminum (DSC) and platinum (TG/DTG) crucibles with approximately 2 mg of the sample. The instruments were previously calibrated and/or verified using the standard calcium oxalate for TG (purity 99.99%) and indium metal for DSC. The analyses were carried out in triplicate.

2.3.2. Karl Fischer titration

The moisture contents of HA, β-CD and PM, PC, FD, FI in the molar ratios 1:1 and 1:2 were determined through the Karl Fischer Titrino Plus KF 870 method (Metrohm). The analyses were carried out in triplicate.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of HA, β-CD and PM, PC, FD, FI in the molar ratios 1:1 and 1:2 were obtained in the range from 4000 to 500 cm^{-1} in KBr pellets using a Shimadzu IRTracer-100 Fourier Transform spectrophotometer at room temperature.

2.3.4. Scanning electron microscopy (SEM)

Samples of HA, β-CD and PM, PC, FD, FI in the molar ratios 1:1 and 1:2 were mounted on aluminum stubs, metallized with gold beams and viewed in a scanning electron microscope (JEOL JSM-6390- model LV) of 8 kV accelerating voltage.

2.3.5. Nuclear magnetic resonance (NMR)

^{1}H spectra H-H spatial dipolar correlation 2D NMR analysis was recorded for the FD preparation in the molar ratio 1:2 dissolved in D_2O (0.02 M) using a Bruker AVANCE 400 spectrometer. Two-dimensional rotating-frame Overhauser effect spectroscopy (2D ROESY) experiments were performed using the standard pulse sequence found in the Bruker pulse program library, applying a set of mixing times of 500, 300 or 150 ms under spin lock condition. During the acquisition, 256 increments were collected with 32 repetitions and the measured data matrix was processed as a matrix of 2k (F2) by 1k (F1) data points. Chemical shifts were measured relative to the peak at 4.80 ppm, due to the solvent (D_2O).

2.3.6. Molecular docking

The starting coordinates for the β-CD were obtained from the X-ray crystal structure of a β-amylase/β-CD complex (Protein Data Bank code: 1BFN), determined at 2.07 Å resolution. The HA and carbohydrate chains structures were first treated by semi-empirical theory at level PM3 using the ArgusLab® v. 4.0.1 program. The optimized structure with the lowest energy was used for the molecular docking calculations. The MGL tools 1.5.6 with AutoGrid4 and AutoDock4 were used to set up and perform blind docking calculations between the ligand and the β-CD. β-CD (as a rigid molecule) and the HA (as a flexible ligand) with files provided using AutoDock Tools®, which means that all non-ring torsions were maintained. Initially, the water molecules were deleted and hydrogen atoms were added into the β-CD structure. Then, the partial atomic charges for the HA and β-CD were calculated using the Gasteiger-Marsili and Kollman methods, respectively. The β-CD was put inside a grid box of 15 Ångström in the x × y × z axis, respectively. The guest was then docked into the β-CD cavity by using AutoDock Vina®. For each of the docking cases, the lowest energy docked conformation, according to the AutoDock Vina®